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# The Effect of Vitamin D. Supplementation on the Growth and Development of Swine

Darwin Eugene Stolte

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THE EFFECT OF VITAMIN D SUPPLEMENTATION ON  
THE GROWTH AND DEVELOPMENT OF SWINE

By

Darwin Eugene Stolte

A thesis submitted  
in partial fulfillment of the requirements for the  
degree Master of Science at South Dakota  
State College of Agriculture  
and Mechanic Arts

August, 1957  
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**THE EFFECT OF VITAMIN D SUPPLEMENTATION ON  
THE GROWTH AND DEVELOPMENT OF SWINE**

This thesis is approved as a creditable, independent investigation by a candidate for the degree, Master of Science, and acceptable as meeting the thesis requirements for this degree; but without implying that the conclusions reached by the candidate are necessarily the conclusions of the major department.

Thesis Adviser

Head of the Major Department

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D.C.S.



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## INTRODUCTION

The importance of vitamin D for efficient growth and performance of swine has been pointed out by many workers in this and other countries. This vitamin, through its specific action in calcium and phosphorus metabolism and bone growth, is necessary for the production of a sound, well-developed skeleton which is essential to profitable pork production.

The fact that common feeds used in swine rations, especially grains and plant protein supplements, are lacking in calcium, vitamin D and in some cases phosphorus, accounts in part for the appearance of rickets and kindred deficiency symptoms under many practical conditions.

Rickets and related bone diseases are normally abated by sufficient exposure to direct sunlight. However, in instances where swine are confined indoors or do not receive sufficient exposure to direct sunlight, some supplemental feed source of vitamin D is indicated.

The following work was undertaken to determine whether vitamin D supplementation was necessary for swine of this area, and what relation the diet and environment have upon their need for vitamin D. The objectives of the experiments performed and described in this study were two-fold. The primary objective was to study the effect of vitamin D supplementation and sunlight on confined growing pigs; and concurrently, determine if antibiotic supplementation exhibits a vitamin D sparing action in swine, as measured by growth and bone development. The objective of the second experiment was to determine the effect of calcium to phosphorus ratios of the ration on the supplemental need for vitamin D by swine.

## REVIEW OF LITERATURE

In reviewing the literature on swine, in reference to rickets and the antirachitic factor, vitamin D, it was considered necessary to preface it with a brief review of the history, chemistry and physiological effects of vitamin D so that the reader may fully appreciate this vitamin's importance and its relationship to rickets.

### History, Chemistry and Physiological Effect of Vitamin D In Its Relationship to Rickets

The influence of the antirachitic factor, now known as vitamin D, has been known to exist for centuries. It was first studied experimentally by Glisson (1650) in infantile rickets as early as the 17th century. He was the first to publish an adequate description of the disease and also opened the field to research by introducing the curative effect of sunlight on rachitic individuals.

Cod-liver oil therapy, as a preventative of rickets, was recognized by Schuette (1824) at the Columbia University Medical School. However, the importance of his discovery was not realized until Hopkins (1906) stated that rickets may be caused by the absence of an "accessory foodstuff." This theory was later confirmed by Zilva et al. (1924), who through the use of nearly fat-free diets on animals deprived of sunlight, demonstrated that either fat-soluble vitamins or sunlight was necessary for the prevention of rickets.

Raczynski (1913) confirmed the findings of Glisson (1650) and Schuette (1824) in a study on the part sunlight plays in the etiology of rickets. By the use of littermate puppies under identical environmental conditions, except for exposure to sunlight, and receiving the same nourishment, he demonstrated that the body of the one reared in darkness contained less  $\text{CaO}$  and  $\text{P}_2\text{O}_5$  in the tissues than did the body of the puppy exposed to sunlight daily. The appearance of rickets in the confined animal suggested that the diminution of these mineral elements in the organism was characteristic of rickets and may result from the lack of action of the sunlight in influencing the assimilation of these elements.

Mellanby (1919) produced conditions which he believed to be rickets by confining littermate puppies to a considerable number of faulty rations. By curing the affected animals with animal fats, he was the first to associate a group of fats rich in vitamin A with the prevention of rickets. Funk (1919), on the basis of the possible relationship of the antirachitic factor to fat-soluble vitamin A, postulated that rickets was one of the "vitamine-deficiency diseases."

It was indicated by Hess and Unger (1920) that vitamin A in cod-liver oil was not the effective vitamin. With an adequate intake of vitamin A from cow's milk, infants were still able to contract rickets. This indicated that the fat-soluble vitamin A was not an antirachitic factor as such.

A notable advance in the knowledge of the etiology of rickets came with the work of Sherman and Pappenheimer (1921). They were able to produce experimental rickets in rats by the use of a diet low in



phosphorus. By substitution of secondary potassium phosphate for a small part of the calcium lactate in the diet, they could completely inhibit the development of rickets. This was the first acknowledgement of the importance of the level of calcium and phosphorus in the diet on the metabolism of these elements so necessary for normal bone growth. McCollum et al. (1921) confirmed these findings by producing experimental rickets in rats on a diet containing an excess of calcium decidedly less than the optimum amount of phosphorus and very little of the organic substance which was especially abundant in cod-liver oil. It was concluded from these observations that certain faulty relations between three dietary factors: calcium, phosphorus and an unidentified organic substance lead to the development of rickets in young rats.

Definite proof that the antirachitic vitamin was an entity distinct from vitamin A was demonstrated by McCollum et al. (1921a). By destroying the vitamin A content of cod-liver oil with a stream of air bubbles at the temperature of boiling water, they observed that the oxidized cod-liver oil was still effective in healing the lesions of rickets.

The action of sunlight in the cure of rickets was not fully appreciated until the publishing of a study by Huldschinsky (1919). He proved, on the basis of x-ray studies, that severe rickets in children could be cured by the light of a mercury vapor quartz lamp. Hess and Unger (1921) demonstrated that sunlight alone possessed the same curative effect on rickets as the light of the quartz mercury vapor lamp. In another study, Powers et al. (1922) concluded that the effect of sunlight and cod-liver oil on growth and calcification of the skeleton appeared to be identical.

Rats were fed on a diet high in calcium, low in phosphorus and insufficiently supplied with the unidentified fat-soluble substance. Both sunlight and cod-liver oil appeared to raise the efficiency of the body cells and enable the organism to put into operation regulatory mechanisms which otherwise would have been ineffectual in the utilization of calcium and phosphorus.

Steenbock and Black (1924) showed that rat rations could be activated by irradiation with the quartz mercury vapor lamp, making them growth-promoting and bone-calcifying to the same degree as when rats were irradiated directly. This discovery came at a time of great benefit to the field of nutrition, as Dunn (1924) discovered that the antirachitic potency of the all important cod-liver oil deteriorated or disappeared after storage. Hess and Weinstock (1925) and Steenbock and Black (1925), in confirming the antirachitic effect of irradiated rations, indicated that the sterol fraction of foodstuffs could be made antirachitically active by irradiation although it was not active itself.

McCollum (1925), on the basis of the previous knowledge of the fat-soluble antirachitic factor and the "vitamin theory" as postulated by Funk (1919), classified the unknown organic substance as a fat-soluble vitamin and named it "vitamin D."

The chemistry of vitamin D is based on the knowledge of the parent or provitamin substances, the sterols. In reviews by Fieser (1937) and Bills (1935), it was pointed out that the sterols have been found to be present in practically all plant and animal tissues, the most important of them being cholesterol.



The next stage in the knowledge of the structure of vitamin D was the discovery that cholesterol itself was apparently not the provitamin. On further study, Pohl (1926) found the material providing the provitamin to be ergosterol, for when it was irradiated, the result was an antirachitic product of great potency. This product was named calciferol or  $D_2$  by Windaus et al. (1932).

Windaus, et al. (1935) prepared pure 7-dehydrocholesterol which on irradiation yielded a highly active form of vitamin D thereafter referred to as  $D_3$ . Vitamin  $D_3$  has a ring structure and conjugated double-bond system the same as calciferol, the difference in the two being in the side chain. Calciferol has the side chain of ergosterol and  $D_3$  that of cholesterol.

Subsequently other workers have discovered several substances having antirachitic properties, which are also classed with the vitamins D; however, the forms  $D_2$  and  $D_3$  are the only ones to have shown any importance in animal nutrition. Hereafter in the manuscript reference to vitamin D shall infer the antirachitic factor as such unless otherwise stated.

There are numerous reports in the literature which indicate that these forms of vitamin D vary in efficiency for certain species of animals. Bethke et al. (1933) reported that the form found in irradiated ergosterol or irradiated yeast,  $D_2$ , was less efficient for chickens than the form found in cod-liver oil or irradiated animal sterol,  $D_3$ . These results have been confirmed by Russell et al. (1933) and Steenbock and Kletzien (1932). It was established by Waddell (1934) and Bethke et al. (1937)

that the vitamin D activity of irradiated cholesterol and cod-liver oil are comparable in efficacy for chickens.

While it cannot be said there is any clear explanation of the physiological action of vitamin D, as cited in the review of literature, many new facts are available which have brought final understanding perhaps a little nearer.

Probably one of the earliest attempts to explain the action of vitamin D on any specific basis was that of Gerstenberger (1933). From clinical observations, he suggested that the liver was involved in the physiological action of vitamin D. It was suggested that the antirachitic factor increased the secretion of gastric juice, thereby facilitating the absorption of calcium. However, Metz and Copens (1934) disclosed that mammalian liver does not contain large amounts of the vitamin as such.

Reed et al. (1939) contended that the provitamin in the skin was eleidin, a sterol secreted by the sebaceous glands, and that activation took place on the surface followed by absorption of the vitamin.

There is strong evidence that vitamin D acts by stimulation of the parathyroid glands, as shown by Albright and Reifenstein (1948). They showed that, in a patient with idiopathic hypoparathyroidism, the administration of large doses of vitamin D increased urinary calcium and phosphorus excretion more than could be accounted for in the decrease in fecal calcium and phosphorus excretion due to the abnormal activity of the parathyroids.

Recent studies with the use of radioactive calcium definitely indicate that vitamin D increases the absorption of calcium as shown by Lindquist (1950). Through a tracer study of the radioactive  $\text{Ca}^{45}$ , it was also shown that the administration of vitamin D promotes mineralization in rachitic bones. Carlsson (1952), with the use of  $\text{Ca}^{45}$  in animals deficient in vitamin D, demonstrated that vitamin D supplementation brought about a rapid removal of the  $\text{Ca}^{45}$  from the formed bone. It was concluded that apparently vitamin D is able to release calcium and phosphorus from certain parts of the bone and make them available for bone growth and cartilage calcification.

The level of vitamin D in the diet has an important bearing on the degree of absorption of calcium and phosphorus, and thus on the blood levels of both of these elements. Hess and Gutman (1922) demonstrated that the sunlight not only brought about a clinical cure of rachitic lesions in infantile babies, but also brought about an increase in the inorganic phosphate of the blood. Steenbock et al. (1923) disclosed that the administration of cod-liver oil would restore the phosphorus and calcium levels of the blood of rachitic rats to normal and increase the ash content of the bone as well.

The importance of blood changes as criteria of metabolic effects of vitamin D was amplified more recently by Shohl et al. (1932). These workers found when the levels of both calcium and phosphorus are raised or lowered in the ration, (the ratio between them remaining the same), the ricket-producing quality of the diet are diminished or increased respectively and the level of blood calcium varied proportional to the

amount in the ration. Thus, at each level of calcium, a different ratio was necessary to produce rickets.

The recent discovery of antibiotics has opened the field of research to new studies relative to the physiological effects of vitamin D. Investigations by Ross and Yacowitz (1953) indicated that penicillin decreased the vitamin D requirement for normal bone calcification in chicks. This action was explained as resulting from the vitamin sparing effect of the antibiotic. Lindblad et al. (1953) also reported a sparing effect of antibiotic on vitamin D. These latter workers found that the growth responses of chicks and turkey poults to chlortetracycline were greater on the lower levels than on the higher levels of calcium, which indicated an improvement in calcium absorption. Jukes (1955) observed an increase in bone ash of chicks fed penicillin at suboptimal levels of vitamin D, indicating that the antibiotic reduced the supplemental requirement of vitamin D for bone calcification.

#### The Relationship of Vitamin D to Rickets in Swine

"Rickets may be thought of as a failure in the metabolism of the animal to deposit the calcium salts which are necessary for complete ossification of growing bone."<sup>1/</sup>

The clinical symptoms of rickets in swine are reviewed by Morrison et al. (1956). The most characteristic symptom is a stiffness of the legs, usually accompanied by a general unthrifty appearance and failure to make good gains in weight. Often paralysis of the hind legs will

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<sup>1/</sup> Bohstedt, G., R. M. Bethke, W. L. Robison, and B. H. Edgington. Mineral and Vitamin Requirements of Pigs. Ohio Agr. Exp. Sta. Bul. 395:64. 1926.

occur, which is due in some cases to a fracture of one of the vertebrae and a resulting crushing or constriction of the spinal cord. Bohstedt et al. (1926) reported that in severe cases of rickets, pneumonia is a common sequel accounting for many deaths.

Steenbock et al. (1924) were the first to report that direct sunlight, by exerting antirachitic properties, was a factor in swine nutrition and thus established that vitamin D was essential to pork production. These conclusions were confirmed by Maynard et al. (1925) who through experiments conducted on littermate pigs on a diet low in calcium, 0.096%, noted that sunlight had a marked influence on the utilization of calcium in the bone. Pigs fed the same ration, but deprived of sunlight, developed a characteristic stiffness within 4 months, and the bones showed severe pathological conditions. These same workers (1925a) reported that cod-liver oil corrected and alleviated poor bone development and stiffness which frequently occurred in pigs fed rations containing an abundance of calcium and phosphorus. The seasonal variation in the conduct of their experiments indicated that there were more cases of stiffness in the winter months than in summer months. Since there was a definite effect on the skeletal structure in all cases viewed, it was concluded by these workers that the terms "swine stiffness," "posterior paralysis" and "rheumatism" do not fit studies in which effects are shown on the bone, but rather fall in the class "rickets."

Bohstedt et al. (1926) observed that cod-liver oil prevented the deformities of the bones of pigs on a diet low in calcium or low in



phosphorus, but did not prevent osteoporosis in adult animals. The administration of cod-liver oil seemed to be instrumental in removing the calcium salts from the shaft of the bone, and depositing them in the cartilage. Though deformities were thus prevented, the weakened shaft was subjected to "spontaneous fractures."

Confirmation of the seasonal variations of sunlight in its curative effect of rickets as shown by Maynard et al. (1925a) is given in a report by Sinclair (1929). In a study of the intensity of solar radiation, it was found that the maximum range of the spectrum for ultra-violet light is observed in July, while in the winter months the smallest amount of ultra-violet radiation is present.

Many workers have shown that the amount of vitamin D required is influenced by the calcium and phosphorus content of the ration, as well as by the ratio in which these elements are present. In a feeding and carcass-analysis experiment designed to study the specific effect of rations on the growth produced, Forbes et al. (1915) found that rations of cereals alone will not produce normal growth of bone. A ration of corn alone and one of corn and soybeans produced the least bone; whereas, the same rations supplemented by tankage or skimmilk produced the most bone. This was the first indication of the effect of the ration on bone development in swine; it brought to light the inadequacy of the commonly used cereal grain rations. Supplemental evidence for this was supplied by Hart et al. (1914) in a study of the calcium and phosphorus content of farm feeds in relation to the animal requirements. In a comparison of the CaO required for a lean pig to secure an increase of 200 pounds live weight as compared to the CaO intake on the calculated 1200 pounds

of feed required for the gain, major swine feeds (corn, wheat, barley, rye, peas and gluten meal), except oats and wheat bran, showed a deficiency of CaO of from 1.02 pounds for corn to .15 pounds for peas.

Following Mellanby's (1919) brilliant researches, Elliot et al. (1922) showed that swine were liable to develop rickets on a low-calcium diet. In one experiment, these workers fed a ration of oatmeal, bran, blood meal, turnips, potatoes, yeast and vitamin D in the form of cod-liver oil, with and without the addition of a mineral mixture consisting principally of calcium carbonate. The pigs fed the ration without the mineral addition became stiff and developed deformities of the long bones characteristic of rickets. When minerals were added, no stiffness developed and growth was more rapid. The bones of the animals receiving the mineral supplement showed a higher content of calcium and phosphorus than did the bones of the pigs receiving the basal ration only. This work indicated that rickets may appear, even in the presence of vitamin D, if the mineral composition of the ration is not adequate. Zilva et al. (1924) concluded that the production of rickets can in no way be associated with a deficiency of mineral constituents in the diet, specifically calcium and phosphorus. The experiments consisted of pigs exposed to sunlight as compared to pigs not exposed to sunlight, both on a nearly fat-free diet with a calcium to phosphorus ratio of 1:0.6. This conclusion was inferred from the development of rickets on the diet containing a 1:0.6 ratio which was supposedly not favorable to rickets. However, a level of 0.6 per cent phosphorus in the ration was later shown by Dunlop (1935) to be the line of demarcation between adequate and inadequate levels of phosphorus. This may account in part

for the results of Zilva and workers, as their rations were considerably lower than the 0.6 per cent level in phosphorus.

Senior (1940) found rickets among young weaned pigs never exposed to sunlight or given vitamin D. This diet carried calcium to phosphorus ratios ranging from 0.76:1 to 1.53:1, and with phosphorus as 0.78 per cent of the ration. Their dams had access to sunlight during pregnancy and lactation. The development of the rachitic condition was prevented by the addition of 1 per cent cod-liver oil to the diet.

Against these findings must be set numerous reports in which rickets did not appear when the antirachitic factor was lacking in the diet. Braude et al. (1943) also found no rickets among pigs fed normal diets containing 0.5 per cent or more phosphorus and a calcium to phosphorus ratio of 1.4:1. Similarly, Bethke et al. (1933) have shown that within calcium to phosphorus ratios of 1:1 to 2:1 in rations containing not less than 0.6 per cent phosphorus, satisfactory growth and bone formation took place without vitamin D supplementation. Dunlop (1935) concluded that diets providing 0.6 per cent phosphorus and a calcium to phosphorus ratio of 0.75:1.0 do not need vitamin D supplementation. Henderson (1924) showed that pigs on a satisfactory diet, well balanced with respect to calcium and phosphorus ( $\text{CaO:P}_2\text{O}_5$ --1:1), exhibited no variation in calcium and phosphorus retention, whether confined to darkness, diffused light, or irradiation. However, if the calcium and phosphorus in the diet were badly balanced, ( $\text{CaO:P}_2\text{O}_5$ --1:3), irradiation definitely increased the calcium and phosphorus retention as compared with control animals kept in the dark.

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It is difficult to reconcile these contradictory findings in the literature. Apparently in the absence of known sources of vitamin D growing pigs may or may not develop a rachitic condition. Rickets appear to be a more certain result when the intake of calcium and phosphorus fall below certain minimal values, which do not appear to be consistent in the literature; or when the calcium to phosphorus ratio deviates widely from 1:1. One factor which may be involved is growth rate. The requirements for calcium and phosphorus are obviously closely associated with the live weight gains being made; and, if through restriction of the intake of these minerals the pigs reduce their rate of gain sufficiently, then rickets may not develop. In the majority of the reports in which growth rates are cited, they are below the "expected gains" set by the U. S. National Research Council (1944).

Another factor having a possible effect on the results of studies on vitamin D requirements for swine is the unknown storage of the vitamin in the animal body. It is interesting to note the findings of Johnson and Palmer (1938) in regards to this factor. An experiment was conducted from January to March involving both colored and white pigs on a ration containing 0.65 per cent phosphorus and 1.07 per cent calcium with a calcium to phosphorus ratio of 1.65:1. At no time did the animals have access to sunlight. The data showed that all the colored pigs, having access to sunlight during the fall before being placed under confinement, stored sufficient vitamin D to protect them from rickets for periods of only 4 to 8 weeks. White pigs, however, raised under the same environmental conditions as the colored pigs, were protected for approximately twice as long.

The increasing use of synthetic vitamin D in farm rations raises question concerning the requirements of swine for this vitamin. Also encountered is the problem of whether or not the irradiated products of different sterols, particularly of ergosterol ( $D_2$ ) and 7-dehydrocholesterol ( $D_3$ ), are equally effective in antirachitic potency for swine. The work of Johnson and Palmer (1941) indicated that the form of vitamin D in sun-cured alfalfa hay is effective for swine. Since the form of vitamin D in plant tissue is recognized to be comparable to the form present in irradiated yeast, as shown by Bethke et al. (1933), it was supposed earlier and later confirmed in work by Bethke et al. (1946) that the yeast and cod-liver oil forms of the vitamin are equally effective for swine. It was further established by these workers that the requirement of swine for vitamin D, in order to prevent rickets, is in the order of 90 U.S.P. units per pound of ration. This was established on a ration containing calcium and phosphorus in ratios of 1:1 to 1.5:1.

Blood serum calcium and phosphorus levels, bone ash and bone breaking strength have been shown by previous workers to be valuable criteria in the study of rickets in swine. Bohstedt et al. (1926) noted differences in the constituents of the blood of pigs when the calcium and phosphorus ratio varied greatly in the rations fed. Loeffel et al. (1931) stated that the blood plasma of animals receiving the direct rays of the sun was higher in calcium and phosphorus than those maintained in the absence of direct sunlight. Hughes (1936), at the California station, reported that the serum calcium and inorganic phosphorus of the young pig was higher than for the mature hog. Also, as the serum calcium

decreased below normal, there was at the same time a fairly regular increase in the inorganic phosphorus in the serum of mature animals, but in young animals there was a decrease. Bohstedt et al. (1926) showed that the addition of 2 per cent  $\text{CaCO}_3$  to a diet composed of white corn, wheat middlings, linseed meal and salt resulted in a higher content of serum calcium in pig's blood than did the basal ration. Dunlop (1935) reported increased calcium and decreased phosphorus in the serum of pigs fed rations containing 1.62 per cent calcium with a calcium to phosphorus ratio of 1:0.34. In studies relating the effect of a low calcium diet on blood serum calcium levels, Bohstedt et al. (1926) reported that pigs fed diets containing less than 0.10 per cent calcium had lower blood serum calcium than those fed rations containing 0.50 to 0.80 per cent calcium. Loeffel et al. (1931) found that pigs fed a basal ration low in calcium had a lower blood serum calcium and inorganic phosphorus than those fed the same ration out of doors. Sinclair (1932) reported that the development of rickets caused a diminution in the serum calcium level without any consistent change in phosphorus levels. The addition of vitamin D exerted an influence in raising the calcium level of the serum to normal. Hughes (1936) reported that the mean serum calcium and inorganic phosphorus levels of the blood of normal pigs of all ages was found to be 11.93 milligrams and 8.34 milligrams respectively.

Forbes et al. (1915) was the first to show that the ash per gram of bone and the breaking strength of the bones varied together in decreasing order as the calcium content of the ration was decreased. Bohstedt et al. (1926) showed that a common sequel of rickets is a diminution of the calcium content of the bones. In all instances, good

bones were not necessarily synonymous with good growth. It was shown that the supplementation of cod-liver oil without the addition of calcium salts to a low-calcium diet caused the development of too brittle a bone which gave way to "spontaneous fractures." Sinclair (1929) reported an increase in the percentage of bone ash and percentage of calcium in the ash when fall pigs were exposed to sunlight. Calcium and phosphorus appeared to be present in the bone in a ratio of 2:1 and in the ration in the ratio of 1.7:1. Bone ash percentages ranging from 58 to 59 per cent were considered to indicate satisfactory utilization and very acceptable bone strength. Similar results were obtained by Dunlop (1935) who concluded that a bone ash content of less than 55 per cent in the dry fat-free bone was the result of an unsuitable diet in relation to calcium and phosphorus or a lack of vitamin D. His study also indicated that there was a positive correlation between blood calcium and bone ash. Bethke et al. (1933a) concluded that a change in the calcium to phosphorus ratio from 0.18:1 to 2.47:1 caused a significant increase in the percentage of ash, whereas, further additions of calcium carbonate with a widening of the ratio to 3.62:1 decreased the ash content and breaking strength of the femurs of growing-fattening pigs. This work indicated that above a certain point, the further addition of calcium and widening of the calcium to phosphorus ratio appears to increase the rachitic quality of the ration.

## MATERIAL AND METHODS

The study reported here consists of two separate trials. One was a preliminary trial where pigs received sunlight, vitamin D and anti-biotic supplementation. In the second trial the pigs received varying levels of calcium and supplemental vitamin D without access to sunlight.

Weanling pigs of comparable age and weight were used in each of the trials conducted. Prior to being put on the experiment, they were immunized against hog cholera and erysipelas and wormed with sodium fluoride.

The experimental animals used in this study consisted of purebred Durocs, Spotted Poland Chinas, Black Poland Chinas and Hampshires. Division into lots was made so as to equalize factors of sex, breed, weight and litter.

The experiment was conducted at the Animal Husbandry Swine Unit located one quarter mile north of the college campus. The pigs were housed and fed in concrete paved lots in the south experimental barn. Ample straw bedding was provided. Each lot was contained in an 8 by 10-foot indoor pen with access to automatic self-waterers at all times. Each of these pens was connected to individual 8 by 16-foot concrete surfaced outdoor runways on the south by means of an upward swinging door (Figure 1). Each lot contained a 200-pound capacity self-feeder for free-choice feeding. Records were kept of all feed consumed by each lot. The feed remaining at the termination of the trial was weighed back and subtracted from the total feed consumed.



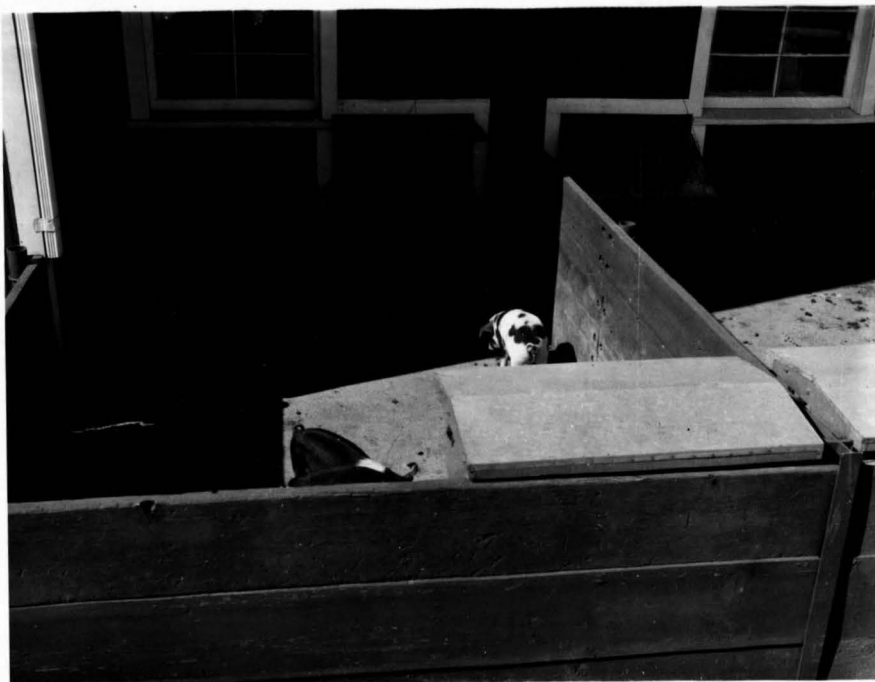


Figure 1. View of Outdoor Connecting Runway

The pigs were weighed individually when allotted to the experiment, and every two weeks thereafter. As they approached 200 pounds in weight, they were weighed weekly, and at 200 pounds they were removed from the experiment. Animals that died or were removed before the trial was completed were autopsied and examined for gross pathological lesions. If the animal had been on the experimental ration for 4 weeks or more, the femurs were removed and preserved by freezing for future physical and chemical analysis.

At the close of the trial, the fastest gaining animal was removed from each lot and slaughtered in the college meat laboratory. The femurs

from each carcass were removed, tagged and packaged and stored in a deep freeze at approximately 28° Fahrenheit for later analyses. In preparation for analyses, they were placed in a forced-air oven for 48 hours at 96° Centigrade to facilitate the removal of adhering cartilage and tissue. Maximum length and smallest diameter of the individually cleansed femurs were determined by means of a micrometer. The weight of each bone was determined by the use of a Ohaus balance calibrated to read to .01 gram.

Breaking-strength determinations were made on a section of each femur shaft. A section of a length twice that of the smallest diameter was removed from the mid-point area of each femur for the determination (Figure 2). Breaking strength was determined with a Tinius-Olsen dynamometer, with inward tension being exerted at both ends of the bone section. This gave an over-all breaking strength reading in pounds, which, when divided by the calculated average cross-sectional area of the section (square inches), gave the strength of the bone section in pounds per square inch.

After the physical measurements had been completed, the entire femur and the removed section were crushed individually in a compressor to facilitate extraction. They were then dried for 24 hours in a forced-air oven at 98° Centigrade, followed by extraction with alcohol and ether to remove the fat and lipoid material. The method varied between trials and is discussed more fully in the detailed presentation of each trial. Following extraction, the samples were dried for 20 hours in a vacuum oven at 60° Centigrade and 35 pounds vacuum.

Duplicate ash determinations were made on a two gram sample of the bone section, and on the entire femur plus the remainder of the

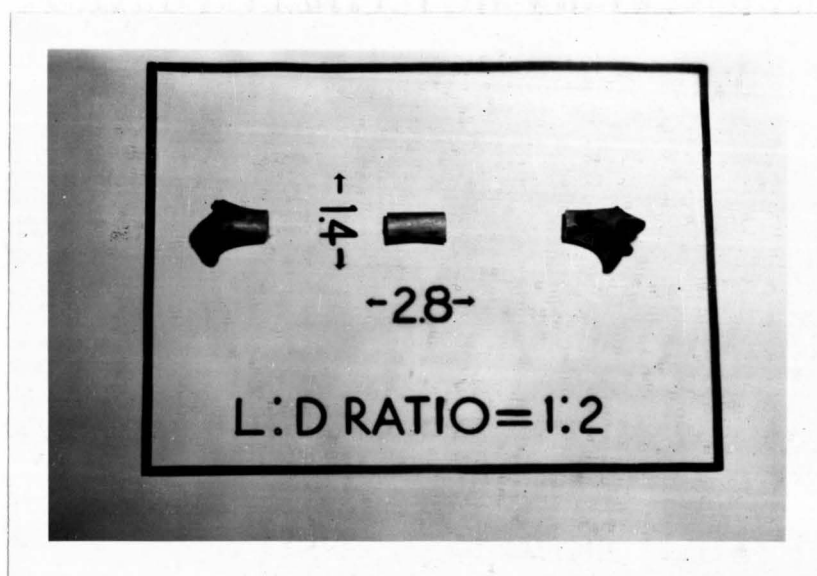


Figure 2. View of Femur Section Removed For Breaking Strength Determination

section. The purpose of the two determinations of each bone was to determine if there was any difference in ash content of a section of the shaft as compared to the entire femur. Calcium and phosphorus determinations were then made on the ashed samples by the Station Biochemistry Department, following A. O. A. C. methods of determination.

The basal rations used in both trials (Table 1) were designed with an optimum calcium to phosphorus ratio of 1.5:1 and containing 15 per cent protein. The experimental rations were mixed on a 1000 pound basis. The irradiated yeast, used as a vitamin D supplement, was added to a premix of ground limestone and trace mineral salt, as were



TABLE 1

Composition of Basal Rations Used  
(Per cent per 1000 pounds ration)

	Trial I	Trial II
Ground yellow dent corn, No. 2	83.10	83.10
Soybean oil meal (44% solvent process)	10.40	10.40
Tankage (60% protein)	5.20	5.20
Ground limestone (37.5% calcium)	.80	.80
Trace mineral salt	.50	.50
2-49C <sup>1/</sup>	.5 lb.	.5 lb.
B <sub>12</sub> Supplement <sup>2/</sup>	.5 lb.	.5 lb.
Zinc carbonate	-----	40 grams

<sup>1/</sup> 2-49C is a vitamin supplement having a minimum guarantee of: Riboflavin 2,000 mg. per pound; Pantothenic acid 4,000 mg. per pound; and Niacin 9,000 mg. per pound.

<sup>2/</sup> The B<sub>12</sub> supplement used carried a guarantee of B<sub>12</sub> activity of 9.0 mg. per pound (L. L. Assay). It was added to the ration, as was the 2-49C, as a preventative against possible vitamin deficiencies.

the other additives of minute amount. They were then thoroughly mixed in a Y-blender before being added to the bulk of the ration. The irradiated yeast used in the two trials was all from the same supply, which was stored under refrigeration for preservation of activity. It was assayed at 18,000 U.S.P. units of vitamin D per gram and was fed at a level of 90 U.S.P. units per pound of ration as recommended by Bethke et al. (1946).

Sunlight, Vitamin D and Antibiotic  
Supplementation For Growing Pigs

The trial was conducted during the summer of 1956. It was designed to determine the effect of supplemental vitamin D and sunshine on confined growing pigs. A study was concurrently made to determine if antibiotic supplementation presented any vitamin D sparing effect in swine.

The experimental animals used in this trial consisted of 24 pure-bred Duroc, Spotted Poland China and Black Poland China weanling pigs which were farrowed in the summer of 1956. They were about 8 weeks of age when placed on the experimental rations and had an average initial weight at the beginning of the trial of 33 pounds.

The trial consisted of six lots of four pigs each, all of which were confined to the indoor pens by closure of the swinging door to the outside runway, except those lots receiving the sunlight treatment. Each of the six lots was fed the Basal Ration I (Table 1), plus the treatment supplementations listed in Table 2. The treatments were randomly assigned to the six lots.

Since both vitamin D and sunlight have been previously shown effective in the prevention of rickets, Lots 2 and 5 were respectively subjected to these treatments. In an attempt to study the effect of antibiotic supplementation on the requirement for vitamin D, Lot 3 was supplemented with antibiotic in the form of aureomycin. Due to the possibility that a joint action of these additives might be involved, Lots 4 and 6 received the vitamin D supplementation in the form of irradiated yeast and each of the two lots received, respectively, the

TABLE 2

Supplemental Treatments Used in Trial I  
(Added to 1000 pounds Basal Ration I)

Lot 1	Lot 2	Lot 3	Lot 4	Lot 5	Lot 6
Basal Ration I	Basal plus 5 gm. Ir- radiated yeast	Basal plus .5 lb. Aurofac- 10 $\frac{1}{2}$	Basal plus 5 gm. Ir- radiated yeast plus .5 lb. Aurofac-10	Basal plus Sunlight	Basal plus Sunlight plus 5 gm. Irradiated yeast

$\frac{1}{2}$  The antibiotic was in the form of Aurofac-10, an anti-  
biotic concentrate containing aureomycin guaranteed  
at 10.0 grams per pound.

the antibiotic and sunlight supplement. The pigs in Lot 1 were used as controls for the trial. Representative samples of the ration from each lot were collected at the mid-point of the experimental period and submitted to the Station Biochemistry Department for analysis. The Basal Ration I analyzed .93 per cent calcium and .47 per cent phosphorus, giving a calcium to phosphorus ratio of 1.97:1, as compared to the designed ratio of 1.5:1. The protein content of the ration on analysis was 15.35 per cent. The trial was of 16 weeks duration--starting the first part of August and terminating in late November.

At the close of the trial, one animal was slaughtered from each lot and the femurs removed for physical and chemical analyses, as previously mentioned. In Trial I, after being cleansed of adhering tissue, the breaking-strength section was removed and a cross-sectional area calculated by measurement with a micrometer. An average of several diameter measurements, taken at 90° angles to each other, was obtained

at both ends of the section and the midpoint. Both inside and outside diameters were calculated at the end points of the section, and an average outside diameter at the midpoint. Following breaking strength determination, the section was sawed in two, laterally, and an inside diameter was taken at the midpoint. Inserting the average diameters, the formula  $\frac{\pi n^2}{4}$  ( $n = \text{diameter}$ ) was used to calculate the inside and outside areas at the three specified points. A net area for each point was then determined by the subtraction of the inside area from the outside area determination. The average of the three net areas gave an approximation of the average cross-sectional area of the bone section. These calculated areas were later subjected to test by the more accurate method of area determination, by use of a planimeter, and were found to be quite accurate regardless of the irregularity of the bone.

In preparation for chemical analysis, the femurs and their corresponding sections were individually partially crushed, and then dried for 24 hours in a forced-air oven at 98° Centigrade. The dried samples were then extracted for 30 hours with alcohol followed by 30 hours with ether to remove the lipid material. Following 20 hours of drying in a vacuum oven, each sample was weighed and then subjected to analysis for total ash, calcium, and phosphorus as previously outlined.

Relation of Calcium to Phosphorus Ratios of the  
Ration on the Supplemental Need for Vitamin D

Previous workers have shown that the ration becomes more rachitic upon the further addition of calcium and the widening of the calcium to phosphorus ratio. This trial was designed to supply calcium to phosphorus ratios of 1.5:1, 2.0:1 and 2.5:1 in order to determine the level at which the ration became rachitic. In order to study the effect of vitamin D supplementation on calcium utilization and deposition, vitamin D was supplemented at the three levels of calcium also. This permitted a comparison within each level of calcium, with and without vitamin D supplementation, as well as between levels of calcium.

The pigs in Trial II consisted of 48 purebred Duroc, Spotted Poland China and Hampshire offspring farrowed in the fall of 1956. Following weaning at about 8 weeks of age, they were allotted by selective randomization into 12 lots of 4 pigs each. The stratification was carried out in such a manner as to allot two Durocs, one Spotted Poland China and one Hampshire to each lot. When placed on the experimental rations, the pigs had an average initial weight of 36.5 pounds.

The trial consisted of six treatments which were replicated. All treatments in this trial received Aurofac-10 in order to increase the growth response. All pigs were confined indoors devoid of direct sunlight throughout the experimental period. The replicated lots were fed the Basal Ration II (Table 1) plus the supplementations shown in Table 3. Basal Ration II (Replicated Lots 1 and 4) served as a control and was designed with an optimum calcium to phosphorus ratio of 1.5:1,



TABLE 3

Supplemental Treatments Used in Trial II  
(Added to 1000 pounds Basal Ration II)

Lot 1 (Rep.)	Lot 2 (Rep.)	Lot 3 (Rep.)	Lot 4 (Rep.)	Lot 5 (Rep.)	Lot 6 (Rep.)
Basal Ration II	Basal plus 6.1 lb. Gr. Limestone <sup>1/</sup>	Basal plus 12.1 lbs. Gr. Lime- stone	Basal plus 5 gm. Ir- radiated yeast	Basal plus 6.1 lb. Gr. Limestone plus 5 gm. Irradiated yeast	Basal plus 12.1 lb. Gr. Limestone plus 5 gm. Irradiated yeast

<sup>1/</sup> Ground limestone was used as a calcium supplement, and was composed mostly of calcium carbonate. It had a guaranteed analysis of 37.5 per cent calcium.

which yielded .725 per cent calcium and .40 per cent phosphorus giving a ratio of 1.81:1. The ration designed for lots 2 and 5 (Replicated), with a ratio of 2.0:1, on analysis contained .92 per cent calcium and .40 per cent phosphorus giving a ratio of 2.33:1. Lots 3 and 6 (Replicated) were designed with a ratio of 2.5:1 of calcium to phosphorus in the ration and analyzed .93 per cent calcium and .38 per cent phosphorus giving a calcium to phosphorus ratio of 2.47:1. As indicated in Table 1, zinc carbonate was added to the Basal Ration II. This was done as a control against parakeratosis which frequently results on diets having a high level of calcium, as shown by Wahlstrom (1957). Zinc carbonate was added to the ration at a level of 40 grams per 1000 pounds of ration and was provided to all treatments at the same level.

The trial was 17 weeks in length--starting October 18, 1956 and terminating February 13, 1957. It was divided into two phases: from

initial weight to 125 pounds and from 125 pounds to 200 pounds. When a lot reached an average weight of 125 pounds, the feed remaining for that period was weighed back, thus enabling the calculation of feed consumption and efficiency for each period of the trial.

Calcium and inorganic phosphorus determinations were made on the blood serum of the individual pigs in Trial II at the end of the experimental period. Each individual animal was placed in the weighing chute for confinement to facilitate the collection of blood. The tail was cleansed with alcohol, clipped free of hair and about a one-half inch slit was made in the tip of the tail. Five to ten milliliters of whole blood were allowed to flow freely into a clean 10 milliter collection tube which was then corked and labeled for analyses. The analyses were conducted by the Station Biochemistry Department, with calcium being determined by the Clark-Collip Modification of the Kramer-Tisdall method and inorganic phosphorus by the Fiske and Subbarow colorimetric determination for blood phosphorus.

Due to the difficulty encountered in making the determination in Trial I, the method of preparing the femurs for analyses in Trial II was varied somewhat. The average cross-sectional area of the femur section, removed for breaking-strength determination, was determined by means of a planimeter. Stamped ink imprints of the end cross-sections were made at the time of removal and of the mid-section following the breaking strength determination. These were then traced with the planimeter, giving a more accurate determination of the area.

Following partial crushing of the femurs and their corresponding sections, they were individually extracted for 15 hours with alcohol followed by 15 hours with ether. They were then dried in the vacuum oven under the same conditions as in Trial I. When drying was complete, they were ground in a Wiley mill using a medium screen to reduce them to a finer, more uniform particle size on which a more accurate chemical analyses could be made. They were then subjected to another 15 hours extraction with alcohol and 15 hours with ether, redried and weighed on a fat-free, moisture-free basis.



## RESULTS AND DISCUSSION

The major criteria considered in this study and the analyses of the results were: rate of growth, feed efficiency, feed consumption and the visual appearance of gross rachitic symptoms. The study later included two additional criteria in an attempt to obtain supplementary information. These were bone development, used to a limited extent in both trials; and the calcium and inorganic phosphorus levels of the blood, which was studied in Trial II only.

### Effect of Sunlight, Vitamin D and Antibiotic Supplementation During the Growing Period

The results concerning the effect of the various supplementations to the feeding value of Basal Ration I are shown in Table 4. It is evident from these data there is no statistically significant difference between the average daily gain and feed requirements per 100 pounds of gain for any of the treatments over the control (Lot 1). These results indicate that there is no need, as measured by growth response, of supplemental vitamin D in confined growing pigs of this area, as was similarly indicated by Hart Steenbock (1922) at the Wisconsin Station. The pigs in Lots 3 and 4, which received the antibiotic supplement, gained approximately 6 per cent faster than did the pigs in Lots 1 and 2. This increase in growth rate is within the range of expectation due to antibiotic supplementation. As a result, there was a reduction in time required to reach market weight by supplementation with the antibiotic. Because a deficiency of vitamin D did not exist, it was impossible to determine if

TABLE 4

A Comparison of Sunlight, Vitamin D and Antibiotic  
Supplementation on Confined Growing Pigs

Supplementation Per 1000# Basal Ration I	Lot 1	Lot 2	Lot 3	Lot 4	Lot 5	Lot 6
	None	5 gm. Ir- radiated yeast	.5 lb. Aur- ofac-10	.5 lb. Aur- ofac-10 plus 5 gm. Irr. Y.	Sunlight	Sunlight plus 5 gm. Irr. yeast
No. of Pigs	4	4	4	4	4	4
No. of Pigs Died or Removed	0	0	0	0	1	0
Av. Initial Wt., lb.	33.2	33.0	33.0	33.0	31.7	33.2
Av. Final Wt., lb.	209.2	203.5	213.8	215.2	198.0	192.8
Av. Total Gain, lb.	176.0	170.5	180.8	182.2	166.3	159.6
Av. No. Days on Feed	99.0	95.0	95.0	95.0	94.3	103.5
Av. Daily Gain, lb.	1.79	1.80	1.90	1.92	1.77	1.56
Av. Daily Feed Cons., lb.	5.70	6.02	5.62	6.34	5.74	5.05
Feed Cons. per cwt. Gain, lb.	321	335	295	331	334	328

the antibiotic exerted a sparing action on the supplemental need for the vitamin. Although it appears that the sunlight supplementation in Lots 5 and 6 caused a reduction in total gain and average daily gain and thus an increase in days required to reach market weight, the variation between Lot 5 and the control (Lot 1) is too insignificant to draw any conclusions. Lot 6, however, varies considerably from both the control (Lot 1) and its correspondingly treated Lot 5. This evidently is due to the individuality of the animals in Lot 6. It is also possible that the results of both Lots 5 and 6 may be credited to the fact that these lots had increased pen space and received more exercise. This indicates that further study needs to be conducted on the effect of sunlight under otherwise identical environmental conditions.

Supplementation of vitamin D to animals receiving sunlight appears to have a depressing effect, though not significant, on rate of gain and to produce a slight increase in feed efficiency as indicated in Lot 6 as compared to Lot 5. This trend in variation may again be due to the individuality of the animals in Lot 6.

Disregarding Lots 5 and 6, due to their difference in environmental conditions, from the rest of the treatments it appears that vitamin D supplementation, in the form of irradiated yeast, brings about an increase in feed consumption as indicated by Lots 2 and 4 as compared to Lots 1 and 3, respectively, in which the treatments were otherwise identical. These results correspond to the findings of Johnson and Palmer (1938), who demonstrated that the addition of irradiated yeast to the ration caused an improvement in appetite.

The animals were individually observed for gross rachitic symptoms throughout the experimental period. There appeared to be no indication of rachitic lesions due to any of the treatments. Though there was some appearance of stiffness in two of the experimental animals early in the period, it gradually diminished and disappeared before the completion of the trial; therefore, it was not charged against the treatment. One case of stiffness appeared in Lot 3, receiving the antibiotic supplement. The animal showed signs of inflammation and swelling in one leg on the third weigh date of the experiment. This condition persisted until the sixth weigh date and then disappeared. The second pig affected, was from Lot 5 having access to sunlight, and developed stiffness in the front legs and an abnormal arched back by the third weigh date of the trial. However, by the fourth weigh date his posture had returned to normal, and the stiffness diminished until it disappeared completely by the fifth weigh date. One animal in Lot 5 was removed from the experiment, after weighing on the 82nd day, because of failure to respond normally. Twelve days later it died and on autopsy was found to have leukemia. There was generalized involvement of the lymph nodes and marked enlargement of the spleen and liver with the tumor cell infiltrations. The death of this pig was not credited to the treatment of the lot, and for analyses of the data, the animal was completely removed from the experiment. The removal required an adjustment in feed consumption by the lot, which was made by subtracting the removed animal's estimated feed consumption during the period it was on the trial. This was computed by taking the average pounds feed consumed per pound gain for the lot, and multiplying by the 62 pounds gained by the animal removed.

Figure 3 shows the femurs removed from one animal in each treatment at time of slaughter. Their physical measurements and chemical analyses are summarized in Table 5.

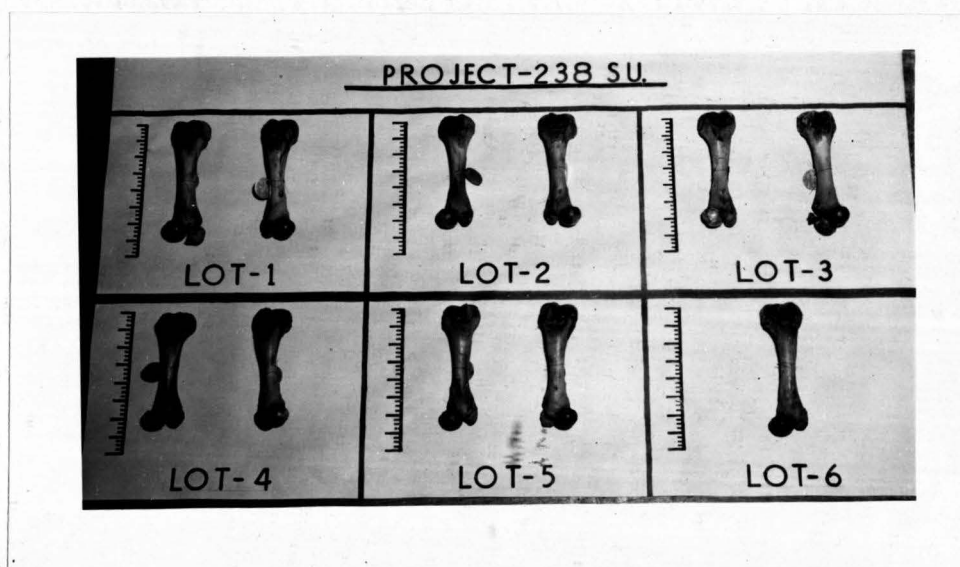


Figure 3. View of the Femur Bones Removed From One Pig in Each Lot of Trial I.

Very little can be concluded from these data because of the limited number of samples. However, various trends are indicative of possible results which may be obtained on further, more complete studies.

As indicated in Figure 3, only one bone was analyzed in Lot 6 because of failure to remove the corresponding bone at time of slaughter. The length and dry fat-free weight of the femurs appear to vary only slightly, with the tendency being toward depressed length and weight



TABLE 5

A Comparison of the Physical Measurements and  
Chemical Analyses of Femurs From Trial I

Supplementations Per 1000# Basal Ration I	Lot 1	Lot 2	Lot 3	Lot 4	Lot 5	Lot 6
	None	5 gm. Ir- radiated yeast	.5 lb. Aur- ofac-10	.5 lb. Aur- ofac-10 plus 5 gm. Irr. Y.	Sunlight	Sunlight plus 5 gm. Irr. yeast
<u>Physical Measurements</u>						
Av. Length, cm.	18.10	17.38	17.92	16.95	18.00	18.60
Av. Dry, Fat-Free Wt., gm.	91.08	89.10	103.50	87.89	92.08	102.20
Av. Breaking Strength, (1000 pounds / sq. in.)	6.73	7.13	7.74	5.66	10.33	12.71
<u>Chemical Analyses</u>						
Ash, per cent						
Section <sup>1/</sup>	72.95	69.42	71.82	72.94	70.01	69.97
Total <sup>2/</sup>	64.28	65.41	64.90	66.14	65.68	64.06
<u>Calcium, per cent</u>						
Section	30.68	31.00	30.24	32.08	33.16	35.20
Total	29.54	26.34	27.81	27.91	25.64	24.75
<u>Phosphorus, per cent</u>						
Section	14.60	13.26	14.27	13.95	12.88	12.85
Total	12.82	11.75	12.95	13.40	10.92	13.36

<sup>1/</sup> The "section" removed for breaking strength determination from which a 2 gram sample was removed for separate analyses.

<sup>2/</sup> The total femur bone plus the remainder of the "section."



of the femurs of confined animals, by supplementation with vitamin D in the form of irradiated yeast, as indicated by comparing Lots 2 and 4 to Lots 1 and 3 respectively. In the lots subjected to sunlight, irradiated yeast supplementation tends to have an additive effect on all physical measurements as indicated by comparing Lot 6 to Lot 5. There also appears to be a significant effect due to sunlight on the breaking strength of the femurs as shown in the data of Lots 5 and 6 as compared to the four confined lots. However, this may in part be credited to the additional exercise these animals received, because of their larger areas of confinement, which may possibly have exerted an influence on bone development.

Due to the inability to produce rickets in the confined pigs fed the basal ration, it is difficult to draw any conclusions from the chemical analyses of the femurs. The bone ash percentages in all treatments fall well above the levels set by Sinclair (1929) of 58 to 59 per cent as indicative of satisfactory utilization and acceptable bone strength. It may be concluded from this there was no rachitic effect on the bone development of any of the treatments. There is very little variation in the results of the calcium and phosphorus determinations. In nearly all lots however, the levels of calcium and phosphorus are higher for the section than for the total femur. The average ratio of calcium to phosphorus in the section is 1.90:1, as compared to 2.16:1 in the total femur. The higher level of these minerals and as a result also total ash in the section indicated that the calcium and phosphorus in the shaft were not needed for normal bone growth and development. This suggested that the

supplemental supply through the ration was sufficient even when vitamin D was not supplemented.

Figure 4 gives a view of the cross-sectional inked imprints of the two ends of the section removed from the mid-point of the shaft.

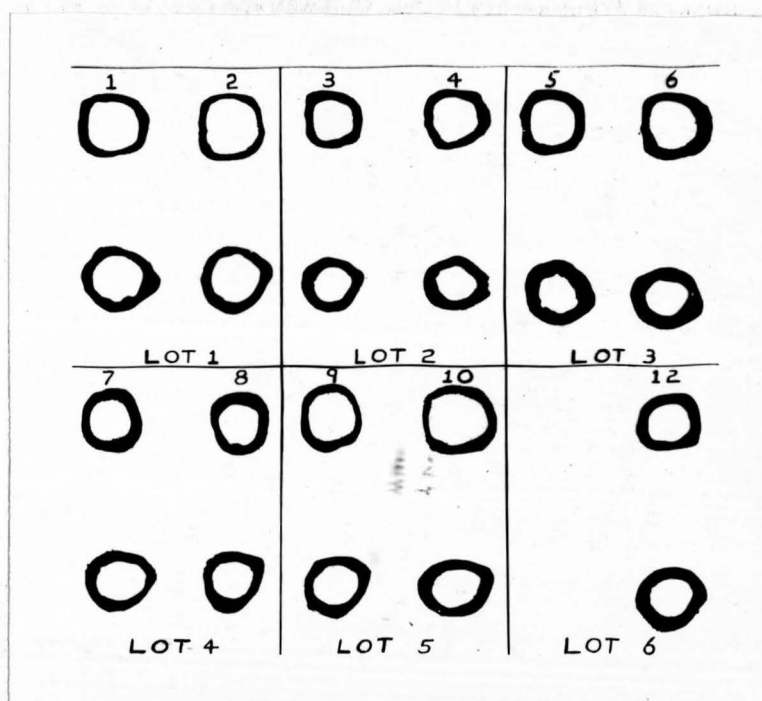


Figure 4. Cross-sectional View of the Ends of the Removed Sections of Each Femur

The only suggestive differences of the cross-sectional view is the apparent thickness of bone structure of Lots 3 and 6. This is confirmed by the additional weight of these femurs, but it is not supported by either increased phosphorus or calcium deposition. These results indicate the variation to be found in the bone formation of various animals, and thus exemplify the inadequacy of the femur results based on only one animal per treatment.

The data obtained from Trial I do not reveal any significant difference between the groups receiving vitamin D supplementation during the growing period and the confined control group, indicating that on a ration well-balanced, with respect to calcium and phosphorus, there is no need for the supplementation of vitamin D.

Effect of Calcium to Phosphorus Ratio of the  
Ration on the Supplemental Need for Vitamin D

A summary of the results of varying the level of calcium in the ration, with and without vitamin D supplementation, is given in Table 6. The weight gain data were analyzed statistically by means of a split-plot design for the purpose of determining the effects due to the interaction of calcium levels and vitamin D supplementation within each level.

It is evident from the data and the analysis of variance shown in Table 7 there is no statistically significant difference between treatments, levels of calcium or the interaction of the two. The data were also analyzed statistically for each phase of the trial to determine if the age of the animals may have affected the response to treatment. In both phases the results were not significant. It appears from these results that increasing the level of calcium in the diet from .725 per cent to .930 per cent, giving calcium to phosphorus ratios of from 1.81:1 to 2.47:1, has no effect on the rate of gain of confined growing pigs. Also, as previously shown in Trial I, the supplementation of vitamin D appears to have no influence on the rate of gain, regardless of the level of calcium present in the ration within the limits used in this experiment.

TABLE 6

A Comparison of Varying Levels of Calcium, With and Without  
Vitamin D Supplementation, Fed to Confined Growing Pigs

Supplementation Per 1000# Basal Ration II	Trt. 1	Trt. 2	Trt. 3	Trt. 4	Trt. 5	Trt. 6
	None	6.1 lb. Gr. Limestone	12.1 lb. Gr. Limestone	5 gm. Irra- diated Yeast	6.1 lb. Gr. Limestone plus 5 gm. Irr. Yeast	12.1 lb. Gr. Limestone plus 5 gm. Irr. Yeast
No. of Pigs per Treatment <sup>1/</sup>	8	8	8	8	8	8
No. of Pigs Died or Removed	2	2	2	2	2	2
Av. Initial Wt., lb.	37.0	37.7	38.5	38.5	38.3	37.7
Av. Final Wt., lb.	204.0	205.2	198.3	201.8	205.7	203.5
Av. Total Gain, lb.	167.0	167.5	159.8	163.3	167.4	165.8
Av. No. Days on Feed	91.8	90.0	90.0	88.7	91.7	93.2
Av. Daily Gain, lb.	1.83	1.86	1.80	1.84	1.84	1.80
Av. Daily Feed Cons., lb.	6.10	6.35	6.31	6.38	6.34	6.34
Feed Cons. per cwt, Gain, lb.	336	341	357	344	347	357

<sup>1/</sup> Each treatment consisted of two replicated lots of 4 pigs each.

TABLE 7

Analysis of Variance Table for the Average  
Daily Gains of Pigs in Trial II

Source	S. S.	D. F.	M. S.
Between Reps.	.001	1	.001
Between Levels	.017	2	.0085
Reps. X Levels	.031	2	.0155
Between Trts.	.001	1	.001
Trts. X Reps.	.034	1	.034
Trts. X Levels	.040	2	.020
Trts. X Reps. X Levels	.159	2	.0795
Remainder	.653	24	.0272
Total	.936	35	

The data are indicative of little variation in the average daily feed consumption of the different treatments, as indicated by the treatment means in Table 6. There appears to be a slight difference in the average daily feed consumption for treatment 1, and as a result a variation in the feed efficiency of this treatment as compared to the others. As was the indication in Trial I, there again appears to be a slight decrease in feed efficiency by the addition of vitamin D, comparing Lots 4 and 5 to Lots 1 and 2. However, because of the variation between the average feed efficiency values for each treatment as shown in Table 8, it is evident that the difference is not significant. An inspection of the mean feed efficiency for each replicate, during each of the two phases of the trial, indicates there is no uniform trend of results. In most cases the variation between replicates is as great or greater than the variation between treatments. There is a definite

TABLE 8

A Comparison of the Mean Feed Efficiency  
Values for Each Replicate of Trial II

Supplementations per 1000# Basal Ration II	Trt. 1	Trt. 2	Trt. 3	Trt. 4	Trt. 5	Trt. 6
	None	6.1 lb. Gr. Limestone	12.1 lb. Gr. Limestone	5 gm. Irra- diated Yeast	6.1 lb. Gr. Limestone plus 5 gm. Irr. Yeast	12.1 lb. Gr. Limestone plus 5 gm. Irr. Yeast
<u>First Phase</u> <sup>1/</sup>						
Replicate 1	259	260	318	302	267	237
Replicate 2	265	289	306	262	305	319
<u>Second Phase</u> <sup>2/</sup>						
Replicate 1	440	395	459	366	416	411
Replicate 2	402	444	353	482	427	487
<u>Total Trial</u>						
Replicate 1	339	331	386	335	336	320
Replicate 2	332	352	327	344	359	393

<sup>1/</sup> The "First Phase" refers to the period including from initial weight to 125 pounds.

<sup>2/</sup> The "Second Phase" refers to the period including from 125 pounds to 200 pounds.



increase in feed consumption per 100 pounds gain during the latter phase of the trial; however, this is a normal result, because of the known decrease in the efficiency of the animal. Of interest are the mean feed efficiencies of treatments 1 and 4. In the first feeding phase, replicate 1 of treatment 1 showed a greater feed efficiency than replicate 2. In the corresponding treatment 4, receiving the vitamin D supplementation, replicate 2 showed the greater feed efficiency. In the second phase, however, the mean efficiencies of the two replicates in each of these treatments were reversed from the above order. The only explanation that can be given for this is the individual differences of the animals in each lot.

The results of the weight gain and feed efficiency data in this trial would indicate that the small differences that may exist between the treatments cannot be determined reliably unless the precision of the experimental design is increased. Possibly individual feeding or increasing the number of replicates per treatment should be considered to give greater statistical control of within treatments, which in the present study may be masking the differences between levels of calcium and between treatments within each level.

Another factor should be carefully considered before any conclusions are drawn from the results of Trial II. This is the removal of one animal from each of the replicated lots, as indicated in Table 6. The reason for the removal in all instances was because of death or failure of the animal to respond. One pig in Lot 2 died on the 43rd day of the experiment and on autopsy was found to have severe gastroenteritis. Another animal, in the corresponding replicate, Lot 2a, was removed from

the experiment the 72nd day because of failure to respond normally. This animal was sacrificed, and on autopsy was found to have a slight cirrhosis of the liver. In Lot 3 a pig died the 25th day of the experiment; however, due to its decomposed condition it was impossible to perform an autopsy. On the 26th day of the trial, an animal was lost in Lot 4. On autopsy, he showed anemia with death resulting from edema of the lungs. There was also fluid in the body cavities. In each case of death there was no definite indication of it being due to treatment received, which is further justified by the two cases of early death (25 and 26 days); this hardly being sufficient time for the treatment to have a fatal effect. In all cases of death or removal the animals were of the Hampshire breed. In the later phase of the trial, two other representatives of this breed, from Lots 1 and 5, developed what was thought to possibly be rickets, signified by enlargement and stiffness of the hocks. Both animals were slaughtered at the completion of the trial, and in both members the small bones of the joints appeared to be fused or calcified. Though this may have been credited to the treatment, it was assumed, because it did not appear in other animals of those respective lots, that the predisposing cause was the general condition of the animal, which in all representatives of the Hampshire breed appeared to be on a lower plane than that of other breeds represented. At the completion of the trial the data indicated that in all treatments the Hampshires produced the lowest gains, and in so doing biased the results of each lot. These reasons are the justification for removing the data contributed by the Hampshire pigs from all lots before analysis.

A constructive argument against this procedure would be that the predisposing cause was unknown, and that it may well have been rickets. For this reason further justification for the removal of the animals is presented in Table 9, which gives the breaking strength and chemical analyses of the bone and level of blood calcium and phosphorus, when obtained, for four of the removed animals which were affected as previously discussed.

TABLE 9

Data Obtained From the Femurs and Blood of Four  
of the Affected Animals Removed from Trial II

Lot No.	Bone Analyses				Blood Analyses	
	Ash %	Calcium %	Phosphorus %	Breaking Strength	Calcium (mg. / 100 ml.)	Phosphorus
1	70.93	30.86	14.35	10.70	10.40	10.77
2	69.28	27.89	14.26	11.04	-----	-----
2a	69.92	29.21	14.13	9.78	-----	-----
5	69.92	35.46	14.53	13.00	14.30	9.87

These data clearly indicate that although these animals did not respond normally and specifically those from Lots 1 and 5, which developed a stiffness characteristic of rickets, the cause definitely was not rickets. The bone ash, calcium and phosphorus levels are all at about the same level as obtained in Trial I, in which case no rachitic symptoms were observed. The breaking strength is well above normal in all instances, and the level of blood calcium is well above the rachitic level of 8 mg.

per 100 ml. as determined by Dunlop (1935). However, in analyzing the results it must be realized that the removal of these animals possibly had some undetermined effect on the results, and any conclusions drawn from the data available must be modified accordingly.

In all cases of removal, feed adjustments were made, the method used depending on the time the animal had been on the experiment. If it had been on experiment through the first phase of the trial or longer, its consumption was calculated on the basis of the average pounds of feed consumed for the lot per pound of gain, as was the procedure in Trial I. If, however, it was on the treatment only a short period, as in the case of Lots 3 and 4, 2.75 pounds feed per pound of gain was arbitrarily assigned. This is an average figure used for a normal animal of this weight.

As the individual animals remaining on the treatments were removed from the experiment, a sample of blood was collected from each by the procedure outlined previously. These samples were then analyzed by the Station Biochemistry Department for serum calcium and inorganic phosphorus. The results of these analyses are given in Table 10. On statistical analyses, there was no significant difference shown between the mean levels of either blood calcium or inorganic phosphorus, due to treatment or level of calcium in the ration. Also there does not appear to be any indication of trends in the variation due to the levels of the treatments. The variation of the samples forming each mean was as great within as between treatments. This can be accounted for partly in that only one sample was taken from each animal, rather than 2 or 3 samples over the period of the trial. As a result the numbers are too limited

TABLE 10

Mean Chemical Analyses of the Calcium and  
Phosphorus Composition of the Blood

Treatment No.	Supplemental Additions Per 1000# Basal Ration II	Calcium (mg/100 ml)	Phosphorus (mg/100 ml)
1	None	11.79	9.42
2	6.1 lb. Gr. Limestone	13.00	8.81
3	12.1 lb. Gr. Limestone	11.98	6.85
4	5 gm. Irradiated Yeast	14.43	9.15
5	6.1 lb. Gr. Limestone 5 gm. Irradiated Yeast	11.74	8.40
6	12.1 lb. Gr. Limestone 5 gm. Irradiated Yeast	12.78	9.34

for reliable results. With the exception of the low phosphorus level in Lot 3, the calcium and phosphorus levels presented are in agreement with the normal levels set by Hughes (1936) of 11.93 mg. calcium and 8.34 mg. phosphorus per 100 milliliters of whole blood. In all treatments the relation of the level of serum calcium to the presence of rickets is in agreement with the results of Dunlop (1935). He found that symptoms of rickets did not appear until the blood calcium value dropped below 8 mg. per 100 ml.

Figure 5 shows the femurs removed from one animal in each treatment at the time of slaughter. Animals were used only from replicate 1 for obtaining femurs, and the chemical analyses of the femur was conducted on the sectional portion only in this trial. The physical measurements and chemical analyses of the removed femurs are summarized in Table 11.



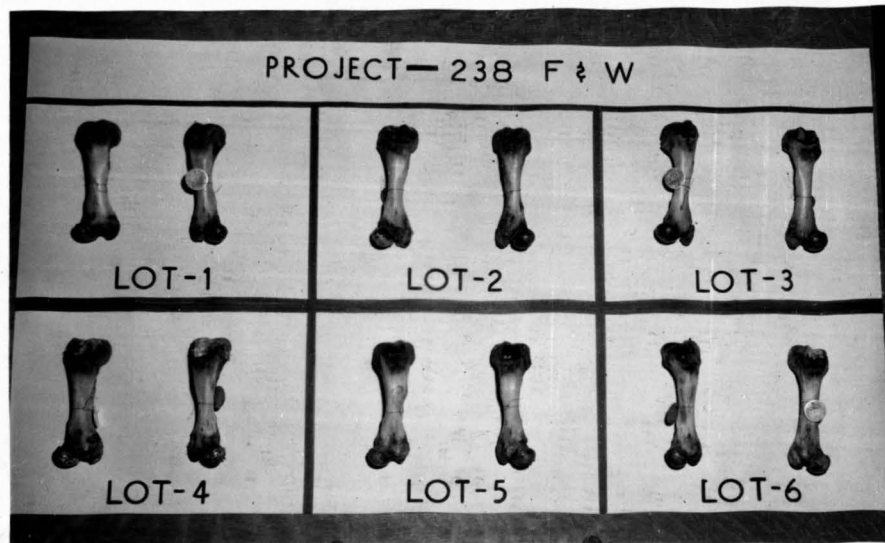


Figure 5. View of the Femur Bones Removed From One Pig in Each Treatment of Trial II.

As in Trial I, only suggestions can be drawn from these data because of the limited number of observations. The average length of the femurs varies only slightly, with no indicative direction as to the variation. There definitely appears to be an effect on the weight and breaking strength of the bone by supplementation with vitamin D as indicated by the increase in weight and strength of the femurs from Lots 4, 5 and 6 as compared respectively to Lots 1, 2 and 3. These results undoubtedly are due to the increase in calcium composition of the bone of the animals supplemented with vitamin D. Confirmation of this is given by the chemical analyses of the femur, which indicates that calcium follows the same trend in all instances. The dry, fat-free weight, breaking



TABLE 11

A Comparison of the Physical Measurements and  
Chemical Analyses of the Femurs From Trial II

	Trt. 1	Trt. 2	Trt. 3	Trt. 4	Trt. 5	Trt. 6
Supplementations per 1000# Basal Ration II	None	6.1 lb. Gr. Limestone	12.1 lb. Gr. Limestone	5 gm. Ir- radiated Yeast	6.1 lb. Gr. Limestone plus 5 gm. Irr. Yeast	12.1 lb. Gr. Limestone plus 5 gm. Irr. Yeast
<u>Physical Measurements</u>						
Av. Length, cm.	17.00	17.60	17.75	17.45	17.30	17.70
Av. Dry, Fat-Free Wt., gm.	104.10	109.98	104.45	107.70	110.02	113.50
Av. Breaking Strength (1000 pounds / sq. in.)	11.88	9.17	9.76	12.20	10.67	11.62
<u>Chemical Analyses <sup>1/</sup></u>						
Ash, per cent	71.96	72.41	71.24	71.85	72.63	72.18
Calcium, per cent	32.01	29.76	29.55	33.43	32.56	32.99
Phosphorus, per cent	14.21	15.15	14.34	14.85	15.12	14.94

<sup>1/</sup> The chemical analyses in Trial II was made only on the "section" removed for breaking strength determination.

strength and per cent calcium appear to indicate that the lower level of calcium used in this trial, (.725%), supplemented by vitamin D in the form of irradiated yeast, makes for a stronger, better developed bone. This is evidenced by comparing treatment 4 to treatment 1 to emphasize the effect of vitamin D supplementation; and to treatment 5 and 6 to emphasize the effect of the level of calcium. It may be concluded from these data that higher levels of calcium in the ration, within the levels used, have no noticeable effect on the development of bone. This suggests that the biological processes of the animal determine the absorption of calcium rather than the level present in the ration. However, there is strong evidence that vitamin D affects the absorption of calcium as indicated by stronger bones containing a higher composition of calcium. None of the treatments approached the rachitic levels as measured by the bone data, indicating that there was no real need for vitamin D supplementation to the confined animals used in this experiment.

Figure 6 gives cross-sectional views of the mid-section and both ends of the removed sections used for breaking strength determination and chemical analyses. There appears to be no suggestive differences between treatments as indicated by the cross-sectional views, other than that Lots 4, 5 and 6 receiving the vitamin D supplementation appear to have a more uniform development of structure than do the non-supplemented lots. At the bottom of Figure 6 are shown the cross-section views of the femurs of the four previously discussed animals removed from the trial because of unknown effects on their response to treatment. There appears to be no indication of weak bone structure in any of these animals.

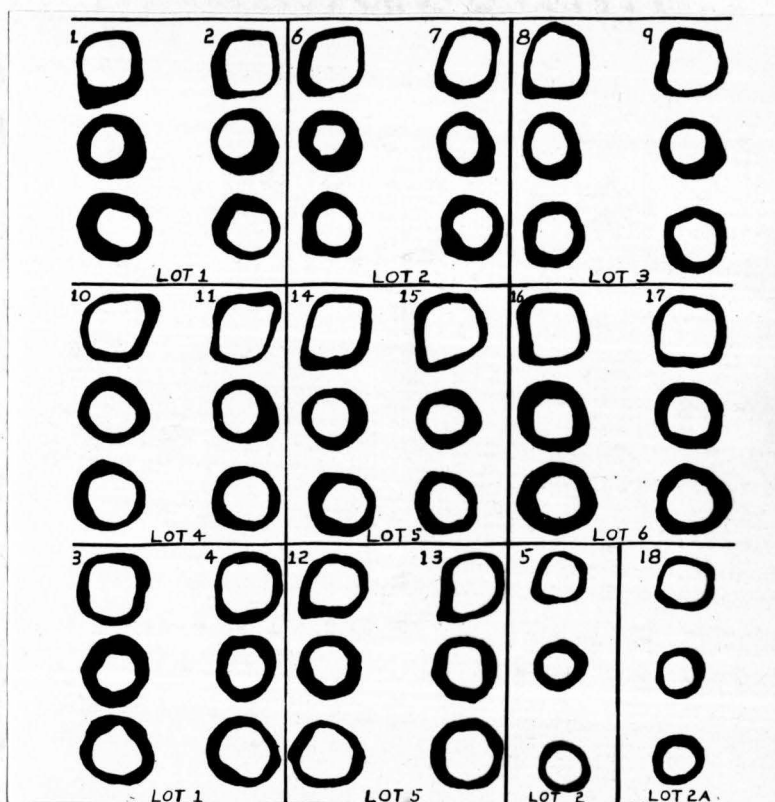


Figure 6. Cross-sectional Views of the Removed Sections of the Femurs in Trial II.

The data from Trial II do not indicate any significant difference between levels of calcium in the ration or supplementation of vitamin D at each level on confined growing pigs, as measured by growth response, blood levels, and bone development. There does appear to be an effect on calcium deposition in the bone when vitamin D is present in the ration. It may be concluded, in summarizing the results, that there appears to be no critical need for vitamin D in confined growing pigs of this area, however, maximum bone development is obtained on a ration containing a calcium to phosphorus ratio of 1.81:1 and receiving vitamin D supplementation.

## SUMMARY AND CONCLUSIONS

An experiment consisting of two separate trials was conducted to determine if there was a need for vitamin D supplementation in practical rations fed to growing pigs in the absence of direct sunlight. The study was concerned with the effect of antibiotic and calcium to phosphorus ratios as they might affect the need for supplemental vitamin D under the conditions imposed.

The first trial, conducted in the summer of 1956, was designed to study the effect of vitamin D and antibiotic supplementation on rate of gain, feed efficiency and bone development of growing pigs. Vitamin D supplementation, in the form of irradiated yeast or exposure to direct sunlight, had no beneficial effect on growth rate or feed efficiency. This fact, together with the absence of rachitic symptoms in all pigs, indicates there was no need for supplemental vitamin D in this trial. There was an increased rate of gain when the antibiotic, aureomycin, was fed to pigs in the absence of direct sunlight. However, it was not possible to determine if the antibiotic exerted a sparing action on the supplemental need for vitamin D as a deficiency of the vitamin did not appear to exist.

In the second trial, carried out in the fall and winter of 1956-57, three different calcium to phosphorus ratios were used to provide varying levels of calcium to the ration. Each level was fed with and without vitamin D supplementation, in the form of irradiated yeast, to determine if the level of calcium in the ration affected the need for supplemental vitamin D. Again it appeared that there was no need for vitamin D supplementation for growth in the absence of direct sunlight, due to the



absence of rachitic lesions. The level of calcium in the diet failed to indicate any effect on rate of gain, feed efficiency, level of blood calcium and inorganic phosphorus, or bone development. Supplementation with vitamin D, although showing no apparent effect on rate of gain, appeared to increase the breaking strength and moisture-free weight of the femur. This was probably due to the noticeable increase in calcium deposition in the bone resulting from supplementation with vitamin D.

In summarizing, it was concluded that although vitamin D supplementation appears to increase bone development and calcification, the experiment did not indicate a supplemental need for the prevention of rachitic lesions in growing pigs of this area confined in the absence of direct sunlight. Also, there was strong indication that within the levels of .725 to .930 per cent calcium used in the diet, the level of calcium had no apparent effect on the need for supplemental vitamin D.

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